Pest Risk Assessment: Importation of Adult Queens, Package Bees and Germplasm of Honey Bees, *Apis mellifera* L., From Australia

Qualitative, Pathway-Initiated Pest Risk Assessment

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I. Introduction

The Act of August 31, 1922, entitled "An Act to regulate foreign commerce in the importation into the United States of the adult honey bee (*Apis mellifica)*" (referred to hereinafter as the Honeybee Act of 1922), prohibits the entry of honey bees from countries where diseases and parasites are known to exist that endanger the health of honey bees. Additional amendments and regulations, promulgated by the Department of Agriculture, extended the Act to prohibit the importation of all life stages of the genus *Apis*, expanded the prohibition to prevent the entry of diseases and pests that endanger the health of honey bees and undesirable germplasm. Regulations promulgated under the Honeybee Act are published in Title 7 CFR Part 322.

The diseases, pests and germplasm specifically identified in the Honeybee Act and amendments, including regulations under the Federal Plant Pest Act entitled Exotic Bee Diseases and Parasites (Title 7 CFR Part 319.76) are as follows:

Exotic Bee Parasites:

Acarapis woodi
Varroa jacobsoni
Tropilaelaps clareae
Euvarroa sinhai
Coelioxys spp.
Chrysis spp.

Exotic Bee Diseases:

Aspergillus spp.
Bacillus spp.
Entomophthora spp.
Beauvaria spp.
Cordyceps spp.
Saccharomyces spp.

Because the protozoan *Nosema apis* is widespread in the United States, it is not considered an exotic disease.

Until recently, only the United States Department of Agriculture could import adult honey bees under the rules and regulations prescribed by the Secretary of Treasury and the Secretary of Agriculture. Recent trade agreements (the General Agreement on Tariffs and Trade, and the North American Free Trade Agreement) obligated the United States to consider imports of honey bees from countries where science-based analyses indicate acceptable risk levels and/or adequate risk management tactics. This pest risk assessment was prepared by the Animal and Plant Health Inspection Service (APHIS) and the Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA) to examine the risks associated with the importation into the

United States of adult queens, package bees (adult queens, adult drones and adult workers) and germplasm (semen and ova) of honey bees, *Apis mellifera* L from Australia. The methods we used to initiate, conduct, and report this pest risk assessment are consistent with guidelines provided by the United Nations Food and Agriculture Organization (FAO) and by the Office International des Epizooties (OIE). This document satisfies the requirements of OIE Guidelines for risk assessment (OIE 1998).

II. Risk Assessment

A. Initiating Event: Proposed Action

Australia first requested access of their honey bees to the United States in 1987. That request initiated an informal risk assessment. The current risk assessment follows a formal request made in January 1997 by the Australian government for access to our market. This assessment closely follows in content and time a recently published (December 9, 1999) risk assessment for the importation of live honeybees into the United States from New Zealand (Docket No. 99-091-1). The Australian apiculture pest risk is very similar to that in New Zealand, differing only by the addition of European Foulbrood disease to those diseases and pests found in New Zealand.

Canada has allowed the importation of honey bee queens and package bees from Australia since 1973. In addition, the movement of honey bees from Canada into the United States has not been regulated or restricted since Canada first allowed entry of Australia honey bees. Although much concern was initially raised about the inadvertent import of *Melittiphis alvearius* and half-moon syndrome from New Zealand and Australia into North America, no reports have indicated adverse events in either Canada or the United States.

III. Assessment of Australian Honey Bee Regulations and Surveillance Programs

The Quarantine Act of 1908 and quarantine conditions issued in 1996 provide the legislative basis for Australian honey bee quarantine policy. Quarantine measures are implemented by the Australian Quarantine and Inspection Service (AQIS). To prevent the introduction of bee diseases and pests, commodities that present a significant quarantine risk such as used beekeeping equipment and live bees may only be imported if they meet stringent health requirements and are accompanied with the proper declaration and health certificate from the country of origin. Entry of honey bees into Australia cannot occur until an import permit has been issued by the Manager, Animal Programs Section, AQIS. Importation of live bees is restricted to queen bees and their escorts. The importation of package bees is not permitted. For countries where varroa mite (*Varroa spp.*), tracheal mite (*Acarapis woodi*) and Tropilaelaps mite (*Tropilaelaps spp.*) occur, the health certificate from the country of origin must confirm that bees to be exported to Australia have been treated with an efficacious acaricide for a period of 56 days immediately prior to export. Pre-export inspection is required to confirm that the hives from which bees for export have been sourced are free of visible evidence of the following honey bee diseases and/or pests:

- American foul brood (*Bacillus larvae*)
- European foul brood (*Melissococcus pluton*)
- External acariasis (*Acarapis extermus, A. Dorsalis, A. Vagans*)
- Tracheal mite (*Acarapis woodi*)
- Half-moon syndrome
- Varroa mite (*Varroa spp.*)
- Tropilaelaps mite (*Tropilaelaps spp.*)
- Bee Lice (*Braula spp.*)

Imported bees are collected by a Quarantine Officer at the Sydney Mail Exchange or Sydney International Airport and delivered to the Eastern Creek Animal Quarantine Station.

For importation of queen bees with escorts the queen is introduced into a nucleus hive at the quarantine facility and the original escorts are killed and examined for:

- Tracheal mite (*Acarapis woodi*)
- Varroa mite (*Varroa spp.*)
- Tropilaelaps mite (*Tropilaelaps spp.*)

Nucleus hives are maintained in flight cages while in quarantine. Larvae produced by an imported queen during quarantine may subsequently be released from quarantine subject to the satisfactory completion of examinations (microscopic where necessary) of appropriate numbers of worker bees and brood to verify that exotic parasites and bee strains are not present. Upon satisfactory completion of quarantine requirements, brood frames can then be removed from the nucleus colony and placed into a grafting room where larvae are grafted into plastic queen cells before being released to the importer. The imported queen is destroyed at the completion of the quarantine process due to the possibility of latent infection with exotic parasites, particularly tracheal mite (*Acarapis woodi*).

Domestic movements of honey bees are regulated through state legislation. State authorities are empowered to place movement restrictions on hives infected with notifiable diseases and to destroy affected hives where necessary for disease control. Each state determines the restricted diseases and controls movements from other states. Interstate movements are permitted subject to satisfactory inspection by state government apiary inspectors. Under existing legislation beekeepers are required to notify relevant state government authorities of notifiable diseases such as American foulbrood, European foulbrood and chalkbrood. Western Australia remains free of European foulbrood. Notifiable diseases also include exotic diseases and pests such as tracheal mite (*A. woodi*) and varroa mite (*V. jacobsoni*).

For export of honeybees to foreign countries, state government apiary inspectors are authorized under the Export Control Act of 1982 to perform pre-export inspections. Inspection report details and laboratory results (where necessary) are sent to the regional AQIS Veterinary Officers.

The certifying Veterinary Officer verifies the report and provided the pre-export results and inspections meet the requirements of the country of destination then an export permit and health certificate are issued. Provision exists for prosecution where necessary.

IV. Assessment of Australia Honey Bee Species and Strains

The honey bee, *Apis mellifera*, is not indigenous to Australia and was first imported into New South Wales in 1822 and Western Australia in 1862 (Gibbs and Muirhead, 1998). Australia allows, with proper permits, the commercial importation of *Apis mellifera* from: Austria, Canada, Canary Islands, Czech Republic, Slovakia, France, Germany, Italy, New Zealand, Norfolk Island, Poland, the United Kingdom, U.S., the Newly Independent States of the former Soviet Union, Croatia, Slovenia, Former Yugoslav Republic of Macedonia, Bosnia and Herzogovina, and the Federal Republic of Yugoslovia.

The Africanized honey bee, *Apis mellifera scutellata* and its hybrids are not known to occur in Australia. The Asian honey bee, *Apis cerana* has spread from Irian Jaya into Papua New Guinea and onto Australian islands in the Torres Strait (January 1992). An aggressive quarantine program has contained the Asian honey bee and it has not been introduced into mainland Australia. The Asian honey bees in the Torres Strait are more than 1200 km from the nearest commercial exporter of queen and package bees (Lacey, 1999).

Based on the history of honey bee importations into Australia, the absence of any reports of species other than *Apis mellifera* or of other adverse subspecies or strains, Australian honey bees are considered the same subspecies of honey bees found in the United States.

V. Pest List: Pests Associated with Honey Bees in Australia

Diseases or Pests in Australia	In U.S.	Comments	References
Fungi			
Ascosphaera apis (Chalkbrood Disease)	Yes		AQIS communicate
Bacteria			
Paenibacillus larvae larvae (American Foulbrood)	Yes	OIE List B Pathogen	AQIS communicate
Melissococcus pluton (European Foulbrood)	Yes	OIE List B Pathogen	AQIS communicate
Protozoa			

Nosema apis (Nosema Disease)	Yes	OIE List B Pathogen	AQIS communicate
Viruses			
Sacbrood Virus	Yes		AQIS communicate
Chronic Bee Paralysis Virus	Yes	Not reported in HI[1]	Liu 1991, Furgala and -Mussen 1978, Liu et al. 1987, Bailey and Ball 1991, Bruce et al. 1995----
Kashmir Bee Virus	Yes	Not reported in HI[1]	Anderson 1991,Furgalaand Mussen 1978, Liu et al. 1987, Bailey and Ball 1991, Bruce et al. 1995
Black Queen Cell Virus	Yes		Furgala and Mussen 1978, Liu et al. 1987, Bailey and Ball 1991, Bruce et al. 1995
Cloudy Wing Virus	Yes		CSIRO communicate
Other Mites			
Acarapis dorsalis Morgenthaler	Yes		Morse 1978, CAPA 1991, Delfinado-Baker 1994,
Acarapis externus Morgenthaler	Yes		Morse 1978, CAPA 1991, Delfinado-Baker 1994,
Mellittiphus alvearius	Yes		AQIS, communicate
Noninfectious Conditions			
Melanosis	Yes		
Beekeeping Pests			
Galleria mellonella (L.) Greater Wax Moth	Yes		AQIS communicate

Achroia grisella (F.) Lesser Wax Moth	Yes		AQIS communicate
Braula coeca Bee-louse	Yes	Tasmania only	AQIS communicate

[1]"Not Reported" acknowledges information received from local beekeepers and apiary inspectors on the apparent absence of a pest in a State. However, no data from science-based surveys have been presented or could be found in the scientific literature to substantiate the claims.

VI. List of Quarantine Pests

A. Quarantine significant diseases or pests in Australia (diseases, pests, or adverse species or strains of honey bees that occur in Australia but not in the United States).

NONE

B. OIE List A Diseases in Australia (transmissible diseases which have the potential for very serious and rapid spread, irrespective of national borders, which are of serious socio-economic consequence and which are of major importance in the international trade of animals and animal products)

NONE LISTED BY OIE.

C. OIE List B Diseases in Australia (transmissible diseases which are considered to be of socio-economic importance within countries and which are significant in the international trade of animals and animal products):

1. *Paenibacillus larvae larvae* (American Foulbrood)

This honey bee disease occurs in Australia and the United States, including Hawaii. *Paenibacillus larvae larvae* is a slender rod-shaped bacterium with slightly rounded ends and a tendency to grow in chains. The rod varies greatly in length, from about 2.5 to 5 microns (mm), and is about 0.5 mm wide. The spore is oval and approximately twice as long as wide, about 0.6 by 1.3 mm. Approximately 2.5 billion spores are produced in each infected larva. If the larva has been infected for less than 10 days, the vegetative cells are present, and some newly formed spores may be seen.

American foulbrood (AFB) disease can destroy a colony of bees if left untreated. The disease can occur anytime during the active brood rearing season. Larvae become immune about 72 hours after egg hatch. The most common means by which this disease is transmitted is by beekeepers who interchange brood combs between healthy and infected colonies. In addition, AFB can be transmitted colony-to-colony by adult bees and also by feeding healthy colonies honey from colonies with AFB. This disease is considered an economic pest and methods to mitigate this vary from country to country and state to state. In most jurisdictions bee inspections program, as we know them today, had their beginnings to mitigate AFB.

Possible sources of disease transmission: queens, package bees (artificial swarms), established colonies with combs, used beekeeping equipment, honey, and pollen.

The disease is detected by inspection of colonies during the brood rearing season. In the U.S., health certificates are traditionally issued by the state inspection services certifying a disease-free source apiary, date of last inspection and inspectors name. No practical method is available for certifying the absence of *Paenibacillus larvae larvae* in package bees and queens.

2. *Melissococcus pluton* (European Foulbrood Disease)

European Foulbrood disease (EFB) occurs in Australia and the United States, including Hawaii. *Melissococcus pluton* is the bacterial causative agent for European Foulbrood disease. The disease is not considered a serious disease by most beekeepers. Only larvae less than 2 days old are affected by the disease which usually strikes in mid to late Spring. Infected larvae usually express a varied microflora. The infectious cycle begins when the larva ingests contaminated food and bacteria establish in the midgut and fill up the midgut increasing the food requirements of the larva. Nurse bees may stop feeding the infected larva or eject it from the colony. Those that die in the colony do so in the coiled stage.

European Foulbrood can be detected using a variety of techniques. Long dead larvae appear as a scale in the cell that is more rubbery than the scale produced by American Foulbrood. The brood comb can take on an unusual appearance with scattered uncapped cells among normal capped cells. The cell caps may also appear concaved whereas the healthy cell cap is convex. The brood comb can have a unique sour smell. Lastly, an ELISA test can be used to identify even low levels of EFB.

Treatment to control EFB is usually not needed. A healthy colony can overcome EFB during a good nectar flow. Stressed colonies are the most effected including those that are moved frequently for pollination services. Antibiotics are available to treat the disease, in particular, oxytetracycline is used.

3. *Nosema apis* (Nosema Disease, Nosemosis)

Nosema disease occurs in Australia and the United States, including Hawaii. *Nosema apis* is the protozoan that causes nosema disease. *Nosema apis* spores are large, oval bodies, 4-6 um long by 2-4 um wide. The spores develop exclusively within the epithelial cells of the ventriculus of the adult honey bee. Nosema disease usually manifests itself in bees that are confined; therefore, the heaviest infections are found in winter bees, package bees, bees used for pollination in greenhouses, etc. Since nosema disease occurs worldwide, it was excluded from the Honeybee Act and its movement within the United States is not under statutory control.

The disease reduces the longevity of adult bees and hence can affect the productivity and survival of honey bee colonies. No single symptom typifies nosema disease. Differences between healthy bees and heavily infected bees can be seen by removing the digestive tract and examining the ventriculus. The ventriculus of a healthy bee is straw brown, and the individual circular constrictions are clearly seen. In a heavily infected bee, the ventriculus is white, soft, and swollen, obscuring the constrictions (White 1918). However, positive diagnosis can only be made by sacrificing adult bees from packages or queen cages for microscopic examination. Fecal material of queens can also be examined for the presence of *Nosema apis* spores.

Possible sources of disease transmission: queens, package bees (artificial swarms), established colonies with combs, and used beekeeping equipment.

D. Other Diseases, Pests or Physiological Maladies of Concern

1. Kashmir Bee Virus

Kashmir bee virus (KBV) occurs in Australia and the United States, but is not reported in Hawaii. KBV was first isolated from adult *Apis cerana*, the Eastern honey bee by Bailey and Woods (1977). Since then, KBV has been isolated from *A. mellifera* in Australia, Canada, and the U.S. The KBV found in each of the countries are serologically related but not considered identical. According to Bailey and Ball (1991) "the Australian strains of KBV were associated with severe mortality of adult bees in the field and have also appeared to cause death of larvae." APIS has noted that subsequent research failed to demonstrate a causal association between KBV and mortality in honey bee larvae (Anderson 1991).

Possible sources of disease transmission: queens, package bees (artificial swarms), and established colonies with combs.

Since *Varroa jacobsoni* is not reported in Australia or New Zealand, it is apparent that KBV is primarily transmitted "bee to bee" and does not require mite transmission. However, diagnosis of the virus requires activation of the virus by injecting a suspect suspension in an apparently healthy pupae and observing for symptoms and confirming the presence of the virus serologically.

Although KBV is "not reported" to occur in Hawaii, no valid surveys have been conducted during at least the past decade to scientifically support claims of its absence from the State.

Consequently,KBV is not considered a Quarantine Pest subject to further consideration in this assessment. However, results from future, science-based surveys in Hawaii could cause reconsideration of this pest relative to imports to that State.

2. Chronic Bee Paralysis Virus

Chronic bee paralysis disease is also referred to as the "hairless black syndrome." The virus that causes chronic bee paralysis is widespread and occurs in Australia and the United States, but is not reported in Hawaii. However the disease rarely causes economic damage. Because the susceptibility to the disease is genetically inherited, generally out-crossing bee stocks remedies the situation.

Possible sources of disease transmission are package bees (artificial swarms), established colonies with combs, and queens.

Chronic bee paralysis virus is not easily detected. Although individual colonies may show adult bees with the symptoms of chronic bee paralysis disease, positive confirmation requires serology. This disease is not included in health certificates used for interstate movement of honey bees in the United States.

Although chronic bee paralysis virus is "not reported" to occur in Hawaii, no valid surveys have been conducted during at least the past decade to scientifically support claims of its absence from the State. Consequently, chronic bee paralysis virus is not considered a Quarantine Pest subject to further consideration in this assessment. However, results from future, science-based surveys in Hawaii could cause reconsideration of this pest relative to imports from Australia to that State.

E. Undesirable Species, Subspecies or Strains of Honey bees

NONE

VII. Quarantine Pests Likely to Follow Pathway (i.e., Quarantine Pests Selected for Further Analysis)

Paenibacillus larvae larvae (American Foulbrood) and *Melissococcus pluton* (European Foulbrood Disease) are considered quarantine pests as a consequence of their status as OIE List B pests. However, the occurrence of these diseases throughout the United States negates much of the risk related considerations in evaluating economic importance and likelihood of introduction.

Although *Nosema apis* (Nosema Disease, Nosemosis) also is an OIE List B pest, we do not list it as a quarantine pest for further analysis due to its wide distribution in the United States, and its exemption as an exotic bee disease under the Honeybee Act. Since the movement of *N. apis* is

not under statutory control within the United States, the SPS agreement stipulates that no sanitary measures can be imposed relative to honey bee imports

Although several pests discussed above are reported not to occur in Hawaii, we can find no scientific evidence to support such claims. As a consequence, we have made no special consideration for the State of Hawaii in this assessment. However, the results from future, science-based surveys could cause reconsideration of this assessment relative to that State.

VIII. Economic Importance: Consequences of Introduction

Since *P. larvae larvae* and *Melissococcuspluton* already occurs in the United States, we rate the economic consequences of introducing these pests as low. This overall rating is based on low economic and environmental consequences, despite high ratings for dispersal capabilities, climatic tolerances and host availability.

IX. Likelihood of Introduction

To determine an overall estimate of the likelihood of introduction of *P. larvae larvae* and *Melissococcuspluton* we estimated the following independent likelihoods:

Expected quantity of queens and packages imported annually	Low
Likelihood of occurring in shipments	Low
Likelihood of surviving shipments	High
Likelihood of not being detected at the port of entry	High
Likelihood of moving to suitable habitats	High
Likelihood of finding suitable hosts	High

The " low" estimate for the likelihood of occurring in shipments is the most critical in this pathway. This estimate is based on compulsory inspections, destruction and reporting for bee disease and prevention in Australia. Since the use of antibiotics is allowed, the presence of AFB could be masked in individual colonies. As a consequence, the annual incidence of AFB in Australia could be higher than the 3-4% infection for the colonies in mainland Australia. In comparison, 1977 estimates of disease in the United States where antibiotics are used, range from a low of 0.0% in several states to a high of 4.0% of colonies inspected in Tennessee and

Wisconsin (Smith, 1998; see also discussions in Matheson and Reid, 1992). Australian colonies are also regularly inspected and all colonies with disease symptoms are removed from the production system and not used as a source of bees for export. As a consequence, it is unlikely that any infected honey bees would be included in shipments to the United States.

Based on these considerations, we conclude that the cumulative likelihood of introducing *P. larvae larvae* is low.

X. Conclusion: Pest Risk Potential and Mitigation Measures

Combining the risk ratings for consequences and likelihood of introduction, we conclude that the overall pest risk potential for *P. larvae larvae* and *Melissococcus pluton* is low. Although this pest already occurs in the United States, its listing as a pest of international importance relative to the movement of honey bees requires caution. Apiary inspection programs in the United States also monitor this pest to prevent its movement in interstate commerce. However, the statutory measures for AFB prevention and control in Australia are at least equivalent to those imposed within the United States. Consequently, the inspection and certification program currently used by Australia for honey bee exports to other countries where AFB is endemic and under statutory control are adequate for shipments to the United States.

We found no evidence of adverse species, subspecies or strains of honey bees that would be of concern relative to the importation of honey bee germplasm from Australia. Likewise, we found no viruses or other disease organisms that posed significant risk to the import of germplasm.

We recommend that all queens and package bees exported from Australia to the United States be from apiaries inspected and certified by Australian regulatory officials as:

1. The bees are a product of Australia.
2. The bees are derived from an apiary or apiaries registered and inspected under, and otherwise complying with AQIS regulations
3. The brood combs in the hives from which the bees are derived showed no clinical signs of American foulbrood on the day of collection.

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XII. References

Anderson, D.L., and A.J. Gibbs. 1988. Inapparent virus infections and their interactions in pupae of the honey bee (*Apis mellifera* L.) in Australia. Journal of General Virology 69:1716-1625.

Bailey, L. and B.V. Ball. 1991. Honey bee pathology. 2nd Edition, 193 pp. Academic Press, Inc., London.

Bailey, L., and M.D. Collins. 1982a. Taxonomic studies on *Streptococcus pluton*. Journal of Applied Bacteriology 53:209-213.

Bailey, L., and M.D. Collins. 1982b. Reclassification of *Streptococcus pluton* (White) in a new genus *Melissococcus pluton*. Journal of Applied Bacteriology 53:215-217.

Bailey, L., and A.J. Gibbs. 1964. Acute infection of bees with paralysis virus. Journal of Insect Pathology 6:395-407.

Bailey, L., and R.D. Woods. 1977. Two more small RNA viruses from adult honey bees and further observations on sacbrood and acute bee-paralysis viruses. Journal of General Virology 37:175-182.

Clark, T.B. 1977. *Spiroplasma* sp., a new pathogen in honey bees. Journal of Invertebrate Pathology 29:112-113.

Clark, T.B. 1978a. Honey bee spiroplasmosis, a new problem for beekeepers. American Bee Journal 118:18-19, 23.

Clark, T.B. 1978b. A filamentous virus of the honey bee. Journal of Invertebrate Pathology 32:332-340.

Colin, M.E., J.P. Faucon, A. Giauffret, and C. Sarrazin. 1979. A new technique for the diagnosis of acarine infestation in honey bees. Journal of Apicultural Research 18:222-224.

Crane, E. 1978. The *Varroa* mite. Bee World 59:164-167.

Dall, D.J. 1985. Inapparent infection of honey bee pupae by Kashmir and sacbrood bee virus in Australia. Annals of applied Biology 106:461-468.

De Jong, D., D. De Andrea Roma, and L. S. Goncalves. 1982a. A comparative analysis of shaking solutions for the detection of *Varroa jacobsoni* on adult honey bees. Apidologie 13:297-306.

De Jong, D., P.H. De Jong, and L.S. Goncalves. 1982b. Weight loss and other damage to developing worker honey bees from infestation with *Varroa jacobsoni*. Journal of Apicultural Research 21:165-167.

Delfinado-Baker, M. 1984. The nymphal stages and male of *Varroa jacobsoni* Oudemans - a parasite of honey bees. International Journal of Acarology 10:75-80.

Delfinado-Baker, M. 1988. Incidence of *Melittiphis alvearius* (Berlese), a little known mite of beehives, in the United States. American Bee Journal 128:214.

Delfinado-Baker, M., and K. Aggarwal. 1987. Infestation of *Tropilaelaps clareae* and *Varroa jacobsoni* in *Apis mellifera ligustica* colonies in Papua New Guinea. American Bee Journal 127:443.

Delfinado-Baker, M., and E.W. Baker. 1982. Notes on honey bee mites of the genus *Acarapis* Hirst (Acari: Tarsonemidae). International Journal of Acarology 8:211-226.

Eickwort, G. C. 1997. Mites: an overview. Pgs. 241-250 *In* Morse, R.A. and K. Flottum (eds). Honey bee pests, predators, and diseases. AI Root, Medina, Ohio, USA.

Gibbs, D.M.H. and I.F. Muirhead. 1998. The economic value and environmental impact of the Australian beekeeping industry. A report prepared for the Australian beekeeping industry.

Goodwin, M. and C. Van Eaton. 1999. Elimination of American foulbrood without the use of drugs; a practical manual for beekeepers. National Beekeepers' Association of New Zealand; Napier, New Zealand. 78 pp.

Guzman de, L.I., T.E. Rinderer, and L.D. Beaman. 1993. Survival of *Varroa jacobsoni* Oud. (Acari: Varroidae) away from its living host *Apis mellifera* L. Experimental & Applied Acarology 17: 283-290.

Hung, A.C.F., H. Shimanuki, and D.A. Knox. 1996. Inapparent infection of acute paralysis virus and Kashmir bee virus in the U.S. honey bees. American Bee Journal 136:874-876.

Lacey, M.J. 1999. Identification and application of the aggregation pheromone of *Apis Cerana*. Rural Industries Research and Development Corportation. Sub-Program 3.3 - Honeybee

Matheson, A. 1993. World bee health report. Bee World 74:176-212.

Matheson, A. and M. Reid. 1992. Strategies for the prevention and control of American foulbrood. American Bee Journal 132:399-402, 471-475, 534-537.

Messing, R. H. 1991. Status of beekeeping in the Hawaiian Islands. Bee World 72:147-160.

Michael, A.S. 1957. Droplet method for observation of living unstained bacteria. Journal of Bacteriology 74:831-832.

Office International des Epizooties, 1998 International Animal Health Code. Part 4 Apiaries 4.2.5

Otte, E. 1973. A contribution of the laboratory diagnosis of American foulbrood of the honey bee with a particular reference to the immunofluorescence method. Apidologie 4:331-339.

Pankiw, P., and J. Corner. 1966. Transmission of American foulbrood by package bees. Journal of Apicultural Research 5:99-101.

Peng, Y-S., and M.E. Nasr. 1985. Detection of honey bee tracheal mites (*Acarapis woodi*) by simple staining techniques. Journal of Invertebrate Pathology 46:325-331.

Peng, Y-S., and K-Y. Peng. 1979. A study on the possible utilization of immunodiffusion and immunofluorescence techniques as the diagnostic for American foulbrood of honey bees (*Apis mellifera*). Journal of Invertebrate Pathology 33:284-289.

Pinnock, D.E., and N.E. Featherstone. 1984. Detection and quantification of *Melissococcus pluton* infection in honey bee colonies by means of enzyme-linked immunosorbent assay. Journal of Apicultural Research 23:168-170.

Ragsdale, D.W., and B. Furgala. 1987. A serological approach to the detection of *Acarapis woodi* parasitism in honey bees using an enzyme-linked immunosorbent assay. Apidologie 18:1- 9.

Ragsdale, D.W., and K.M. Kjer. 1989. Diagnosis of tracheal mite (*Acarapis woodi* Rennie) parasitism of honey bees using a monoclonal based enzyme-linked immunosorbent assay. American Bee Journal 129:550-553.

Ritter, W., and F. Ruttner. 1980. Diagnoseverfahren (*Varroa*). Allgemeine Deutsche Imkerzeitung 5:134-138.

Shimanuki, H., and D.A. Knox. 1988. Improved method for the detection of *Bacillus larvae* spores in honey. American Bee Journal 128:353-354.

Shimanuki, H., and D.A. Knox. 1991. Diagnosis of honey bee diseases. USDA, Agriculture Handbook No. AH-690, 53 pp.

Smith, I. Barton, Jr. 1998. 1997 Apiary Inspection Statistics. In Proceeding of the 1998 Annual Conference Apiary Inspectors of America. Lawrence, Kansas. 68 pp.

Szabo, T.I. 1989. The capping scratcher: A tool for detection and control of *Varroa jacobsoni*. American Bee Journal 129:402-403.

Toschkov, A., T. Vallerianov, and A. Tomov. 1970. Die Immunofluoreszenzmethode und die Schnelle und Spezifische Diagnotik der Amerikanischen Faulbrut bei der Bienenbrut. Bulletin Apicole de Documentation et d'Information 13:13-18.

White, G.F. 1912. The cause of European foulbrood. U.S. Department of Agriculture, Bureau of Entomology Circular 157, 15 pp.

White, G.F. 1918. Nosema disease. U.S. Department of Agriculture Bulletin 780, 59 pp.

White, G.F. 1920. European Foulbrood. U.S. Department of Agriculture Bulletin 810, 39 pp.

Zhavnenko, V.M. 1971. Indirect method of immunofluorescence in the diagnosis of foulbrood (American and European) (in Russian). Veterinariya (Kiev) 8:109-111.